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(54) Title: COMBINATORIAL CHEMISTRY SYSTEM AND METHOD OF USE

(57) Abstract

The present invention provides a combinatorial chemistry system and method of use. The system includes a bead holder, equipment for screening of molecules on polystyrene beads, and equipment for characterization of molecules on the beads. The method takes advantage of the system by utilizing a dual process. The dual process allows the screening of the molecules on a library of the beads, while at the same time allowing the characterization of the molecules on each bead. The system and method reduce the time to screen and characterize molecules on beads of a library.

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# COMBINATORIAL CHEMISTRY SYSTEM AND METHOD OF USE

This application claims priority to U.S. Provisional Application Number 60/064,708 filed November 7, 1997.

Combinatorial chemistry is a drug discovery technology being employed by pharmaceutical companies worldwide. Through combinatorial chemistry, a strategy of diversity is used to synthesize as many different molecules as possible and test their reaction to a specific "target", such as a disease or cell structure. Screening is the chemical assay of the molecules with the "target". If any of the molecule(s) show some reaction to the "target" during screening, the molecule(s) become a candidate for a commercial drug. The candidate molecule(s) are then characterized to determine both their composition and structure to enable additional synthesis and testing. One technique of employing combinatorial chemistry utilizes polystyrene beads as a support structure for the molecules to be tested.

The polystyrene beads are small, having diameters ranging from 1000 microns to less than 20 microns. Large numbers of polystyrene beads coated with members of a single class of compound, such as peptides, constitute a collection known as a library. Each library can include thousands of the beads with different molecules, yet each bead contains only a single type of molecule. Processing of the beads to test their reaction to specific "target" requires exposure to a bio reagent indicative of the "target". Each bead is screened to determine if there is any activity between the molecule(s) on the bead and the "target". Any activity between the molecule on a bead and the "target" is considered a "hit" and that molecule is further tested.

The usefulness of the bead technique is limited by the difficulty of manipulating individual beads, screening the molecules and then characterizing the "hits". Presently, a technique of electrospray mass spectrometry is utilized for characterizing the "hits", in which the characterization process can take as long as 15 minutes to complete. Because the screening of a large library may result in the discovery of many thousands of possible "hits", the characterization of large libraries requires huge expenditures of time and labor. This has necessitated the use of smaller libraries, which do not take full advantage of the combinatorial chemistry benefits.

Work by investigators has shown that beads can be characterized much more rapidly using Time-of-Flight/Secondary Ion Mass Spectrometry (TOF/SIMS). TOF/SIMS is another mass spectrometer technique. The TOF/SIMS reduces the time required for characterization of a single bead from 15 minutes to less than 1 seconds. However, the manual labor required to array the beads on a substrate after the screening process has been performed and also keep the beads positioned during the TOF/SIMS characterization has limited the usefulness of this technique.

It is an object of the present invention to provide a system and method to screen molecules on a library of beads, while also characterizing the molecules on the beads.

It is an object of the present invention to reduce the time it takes to screen and characterized molecules on a library of beads.

It is an object of the present invention to provide a bead holder to aid in the above-mentioned objects of the present invention.

## SUMMARY OF THE INVENTION

The present invention is a combinatorial chemistry system and method of use for the dual processing of different molecules coated on a library of beads. The system includes beads coated with different molecules on each bead, a bead holder, screening equipment and characterization equipment. The bead holder retains the beads so that each of the beads has an exposed first section of the bead which is exposed independently of an exposed second section of the bead. The screening equipment and characterization equipment is used to assay and characterize the molecules on each bead. The method of use includes screening the exposed first section of the beads which are coated with molecules and characterizing the molecules located on the exposed second section of the beads. The method utilizes a library of the beads in the bead holder, whereby the bead holder retains the beads as described above. Included in the method is the screening of molecules on the beads, while allowing the characterization of the molecules on beads during the screening process.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of a bead holder according to the present invention;

Fig. 2 is a perspective view of a bead holder according to the present invention;

Fig. 3 is a cross-sectional view of a first embodiment of a bead holder according to the present invention;

Fig. 4 is a cross-sectional view of a first embodiment of a bead holder according to the present invention;

Fig. 5 is a cross-sectional view of a first embodiment of a bead holder according to the present invention;

Fig. 6 is a cross-sectional view of a second embodiment of a bead holder according to the present invention;

Fig. 7 is a cross-sectional view of a second embodiment of a bead holder according to the present invention;

Fig. 8 is a cross-sectional view of a third embodiment of a bead holder according to the present invention; and

Fig. 9 is a cross-sectional view of a third embodiment of a bead holder according to the present invention.

## DETAILED DESCRIPTION

The present invention provides a combinatorial chemistry system and method of use. The system includes a bead holder 10; equipment (not shown) for screening of molecules on polystyrene beads 12; and equipment (not shown) for characterization of molecules on the beads 12. The method takes advantage of the system by utilizing a dual process. The dual process allows the screening of the molecules on a library of the beads 12, while at the same time allowing the characterization of the molecules on each bead 12. The system and method reduces

the time to screen and characterize molecules on beads 12 of a library, while taking advantage of the speed of equipment such as the TOF/SIMS.

The bead holder 10 of the system provides the exposure of two independent surface sections of the overall surface of each bead 12 held by the bead holder 10. The bead holder 10 is a plate 11 which includes holes 18 to receive the beads 12. The bead holder 10 can be of any geometric shape, such as a rectangle 14 or circle 16 shown in Figs. 1-2. The amount and size of the holes 18 of the bead holders 10 shown in Figs. 1-2 are for only illustrative purposes only. An actual bead holder 10 can be quite tiny and have many thousand of holes 18 for receiving the same number of beads 12. The number of beads 12 held by the bead holder 10 is only limited by the size of the holder 10 and the size of the beads 12. The holes 18 constrain each bead 12 to a specific location, but allow removal of the beads 12 for further evaluation and archiving if required. Production of the bead holder 10 utilizes semiconductor etching processes in order to form the holes 18 on a substrate. The thickness of the substrate depends upon the diameter of the bead 12 to be used. The usual materials for the substrate should be of a non-reactive material, such as a non-reactive metal. However, it is conceivable that the substrate could be fabricated from ceramic or glass. Figs. 3-9 show cross-sectional views of different embodiments of the bead holder 10. Each of Figs. 3-9 show only one hole 18 and bead 12 for illustrative purposes. The difference in the embodiments is the type of hole 18 in each bead holder 10. Each bead 12 positioned in the bead holder 10 includes an exposed top surface section 20 and exposed bottom surface section 22.

Figs. 3-5 show a first embodiment of the bead holder 10. The holes 18 of the bead holder 10 of the first embodiment include slanted sides 24 which slant away from each other starting at the bottom 26 of the holes 18. To insert the beads 12 into the holes 18, more beads 12 then holes 18 are layered on a top surface 28 of the bead

holder 10 until all of the holes 18 are filled. A vacuum can be additionally applied from below the holes 18 to cause air or liquid to flow through the holes 18 and pull the beads 12 into each hole 18. The vacuum can also aid in retaining the beads 12 in the holes 18. After all the holes 18 have been occupied, the excess beads 12 are removed with either a rinse, a vacuum chuck or a mechanical brush. A plate 30 is then placed on top of the bead holder 10, as shown in Fig. 4. The plate 30 can be flat (not shown) or have dimples 32 which are aligned to match the holes 18 of the bead holder 10. The size and shape of the dimples 32, depends upon the diameter of the beads 12 used. Pressure is applied from above to either type of plate 30. The pressure from the plate 30 against the beads 12 forces the beads 12 to partially extrude and deform through the holes 18 of the bead holder 10, as shown in Fig. 5. After the plate 30 is removed, the beads 12 are retained by the holes 18 due to a pressure fit.

A second embodiment of the bead holder 10 is shown in Figs. 6-7. The second embodiment uses a two piece substrate construction of a bottom layer 34 and top layer 36. Each layer 34, 36 has a set of holes 18 which align with each other when the two layers 34, 36 are place together. Unlike the first embodiment, the second embodiment retains the beads 12 without forcing the beads 12 to change shape. The shape of the sides 38 of the holes 18 in each layer 34, 36 are for locking the beads 12 in place. Most any geometric shape for the sides 38 can be used, as long as the sides 38 of both layers 34, 36 lock the beads 18 in place. Figs. 6-7 show the sides 38 slanted as an example. To insert the beads 12 in the holes 18, more beads 12 then holes 18 are layered on a top surface 40 of the bottom layer 34 until all of the holes 18 are filled. A vacuum can be additionally applied from below the holes 18 of the bottom layer 34 to cause air or liquid to flow through the holes 18 and pull the beads 12 into each hole 18. The vacuum can also aid in retaining the beads 12 in the holes 18. After all the holes 18 of the bottom layer 34 have been occupied,

the excess beads 12 are removed with either a rinse, a vacuum chuck or a mechanical brush. Next, the top layer 36 is aligned over the bottom layer 34 in order to trap the beads 12, as shown in Fig. 7. The bottom and top layers 34, 36 are either bonded together using adhesive or, if there is the possibility of needing to retrieve the beads 12, the layers 34, 36 can be held together by a clamping fixture (not shown).

Figs. 8-9 show a third embodiment of the bead holder 10. The third embodiment of the bead holder 10 includes holes 18 which are of a diameter that are slightly less than the diameter of the beads 12. As shown in Figs. 8-9, the third embodiment includes a reduction in diameter of the holes 18 near a bottom surface 42 of the bead holder 10, which forms a lip 44. The beads 12 are inserted into the holes 18 from a top surface 46 of the bead holder 10. First, more beads 12 then holes 18 are forced towards the holes 18 in a contained environment until all of the holes 18 are filled. The beads 12 can be forced into the holes 18 using a stream 48 from either an air stream from above the holes 18, a liquid stream from above the holes 18 or a vacuum applied from below the holes 18, as shown in Fig. 8. The lip 44 acts as a stop to restrain the beads 12 from passing through the holes 18. After all the holes 18 have been occupied, the excess beads 12 are removed with either a rinse, a vacuum chuck or a mechanical brush. The beads 12 are retained by the holes 18 due to a pressure fit.

The method of using the system allows use of combinatorial chemistry techniques to reduce the time to screen and characterize molecules on each bead 12 of a library. The method employs the dual process of screening the molecules on one side of the bead holder 10 and at the same time characterizing molecules on the other side of the bead holder 10. For example, molecules on the exposed bottom surface section 20 of each bead 12 in the bead holder 10 can be screened. At the same time that the bottom surface section 20 is being screened, molecules on the top surface

section 22 of each bead 12 can be characterized. Assay equipment known in the art is used to perform the screening and characterization equipment known in the art is used to perform the characterization process. The method allows the use of larger libraries of beads 12 and takes advantage of equipment like the TOF/SIMS. A computer and software can be utilized to control when to screen the molecules on the beads 12; when to characterize the molecules on the beads 12; and whether to characterize the molecules on all of the beads 12 or just the beads 12 with "hits". The combination of the method of use of the system's bead holder 10 and computerization allows the user to take full advantage of the combinatorial chemistry benefits. This method is also desirable because the assaying of beads 12 can cause chemical contamination of the beads 12. Contamination of beads 12 can complicate the characterization procedure. The bead holder 10 of the present invention aids in preventing contamination of part of the bead 12, so that the molecule(s) on the bead 12 can be characterized.

While different embodiments of the invention has been described in detail herein, it will be appreciated by those skilled in the art that various modifications and alternatives to the embodiments could be developed in light of the overall teachings of the disclosure. Accordingly, the particular arrangements are illustrative only and are not limiting as to the scope of the invention which is to be given the full breadth of the appended claims and any and all equivalents thereof.

We Claim:

1. A bead holder for holding beads, said beads coated with molecules to be processed, comprising:

a plate; and

a plurality of holes for retaining said beads, said holes retaining said beads such that a first section of said bead is exposed independently of a second section of said bead.

2. The bead holder of claim 1, wherein said holes of said bead holder include slanted sides so that a bottom open area of said holes is smaller than a top open area of said hole; and wherein said bottom open area is smaller than said bead.

3. The bead holder of claim 1, wherein said bead holder includes an assembled top layer and bottom layer; wherein said top and bottom layers each include holes for retaining said beads; wherein said holes of said top layer align with said holes of said bottom layer when said top and bottom layer are assembled; and wherein sides of said holes of said top and bottom layers are geometrically shaped to trap said beads between said top and bottom layers.

4. The bead holder of claim 3, further including a plate to press said beads into said holes.

5. A bead holder of claim 1, wherein said holes of said bead holder includes a bottom open area of said holes that is smaller than a top open area of said holes; wherein said hole include a lip at said bottom open area for retaining said bead; and wherein said top open area is smaller than said bead.

6. A combinatorial chemistry system for the dual processing of molecules coated on a library of beads comprising:

beads coated with at least one molecule; and

a bead holder, said bead holder retaining said beads so that each of said beads has an exposed first section of said bead which is exposed independently of an exposed second section of said bead.

7. The combinatorial chemistry system of claim 6, further including equipment for screening said molecules on said first section of said beads while said beads are in said bead holder and equipment for characterizing said molecules on said second section of said beads while said beads are in said bead holder.

8. The combinatorial chemistry system of claim 6, wherein said bead holder is a plate, said plate including holes to retain said beads.

9. The combinatorial chemistry system of claim 8, wherein said holes of said bead holder include slanted sides so that a bottom open area of said holes is smaller than a top open area of said hole; and wherein said bottom open area is smaller than said bead.

10. The combinatorial chemistry system of claim 8, wherein said bead holder includes an assembled top layer and bottom layer; wherein said top and bottom layers each include holes for retaining said beads; wherein said holes of said top layer align with said holes of said bottom layer when said top and bottom layer are assembled; and wherein sides of said holes of said top and bottom layers are geometrically shaped to trap said beads between said top and bottom layers.

11. The combinatorial chemistry system of claim 10, further including a plate with dimples to press said beads into said holes.

12. The combinatorial chemistry system of claim 8, wherein said holes of said bead holder include a bottom open area of said holes that is smaller than a top open area of said holes; wherein said hole include a lip at said bottom open area for retaining said bead; and wherein said top open area is smaller than said bead.

13. The combinatorial chemistry system of claim 7, wherein said characterization equipment includes at least a Time-of-Flight/ Secondary Ion Mass Spectrometry.

14. A combinatorial chemistry method comprising:

screening a first section of at least one bead which is coated with at least one molecule; and

characterizing at least one molecule located on a second section of said beads.

15. The method of claim 14, further including a library of said beads to be processed; screening said first section of said beads of the library; and at the same time characterizing said second section of said beads of the library.

16. The method of claim 15, further including a bead holder to retain said beads of the library; said bead holder retaining said beads so that said first section and said second section is exposed; and said first section being exposed independently of said second section while said bead is in said bead holder, so that the processing of one of said sections does not affected said other section.

17. The method of claim 14, wherein said characterization of said second section is performed by a Time-of-Flight/Secondary Ion Mass Spectrometry.

18. The method of claim 14, wherein a computer controls the simultane us screening and characterization of said beads.

19. The method of claim 16, wherein said bead holder is a plate which includes holes to retain said beads.

20. The method of claim 16, wherein said beads are loaded into said bead holder by using a fluid stream to force said beads into said holes.

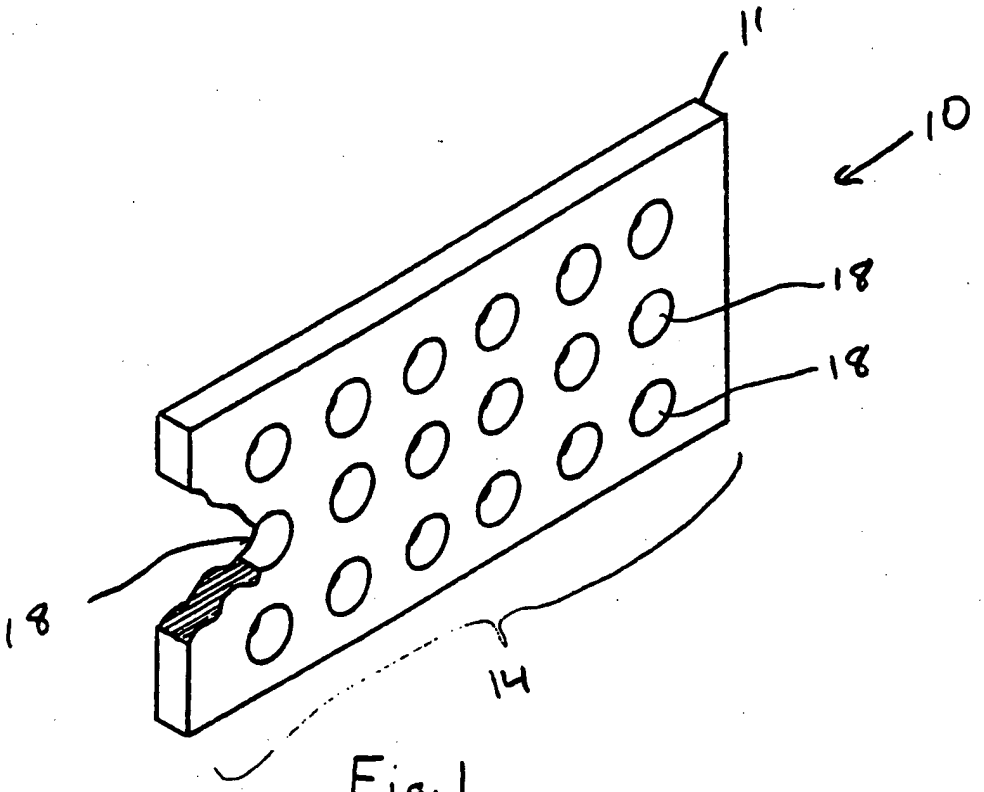


Fig. 1

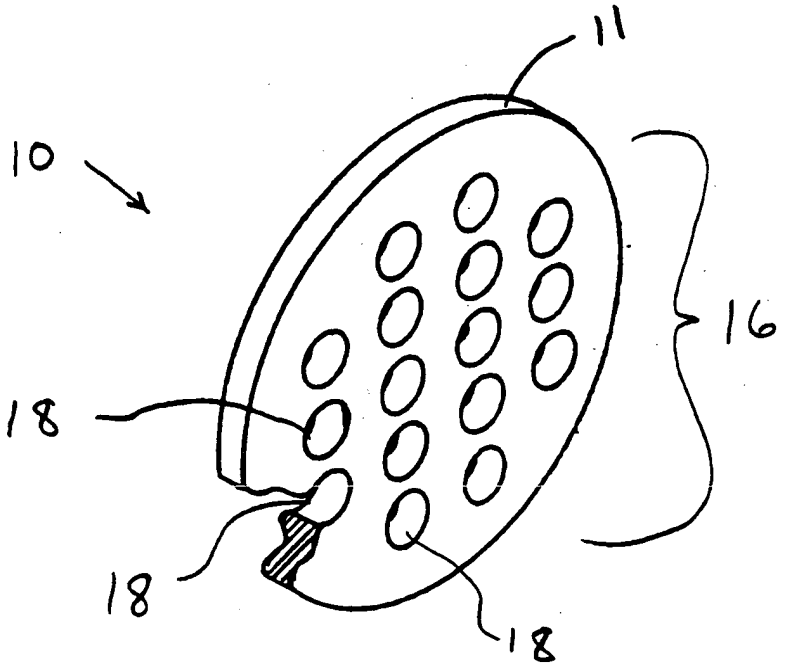


Fig. 2

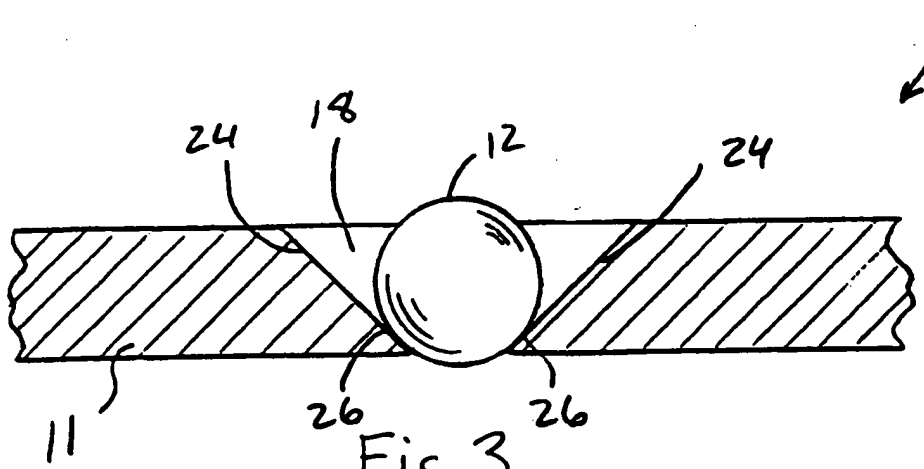


Fig. 3

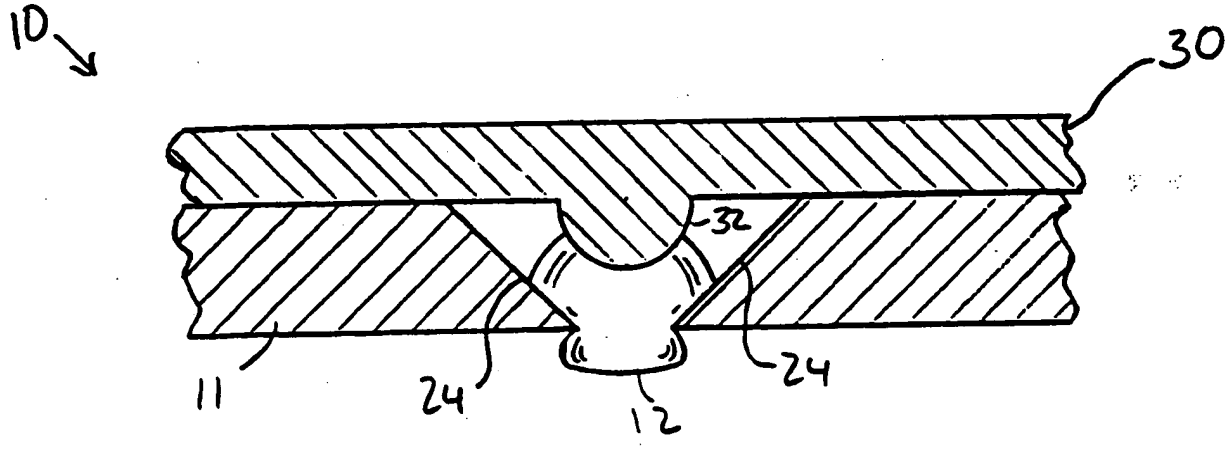


Fig. 4

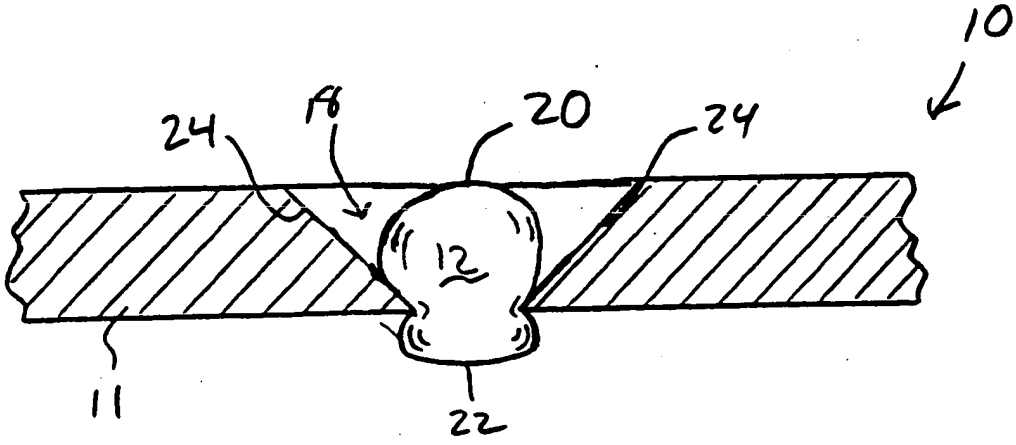


Fig. 5

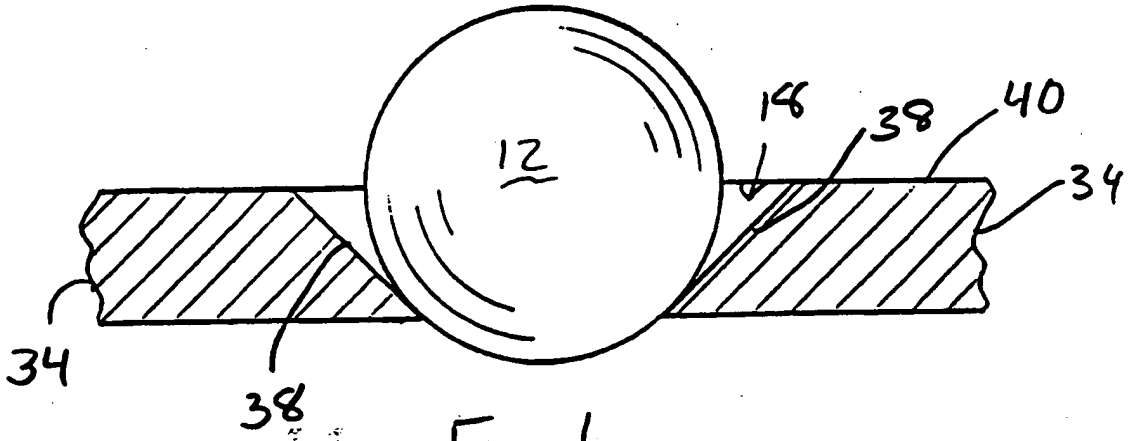


Fig. 6

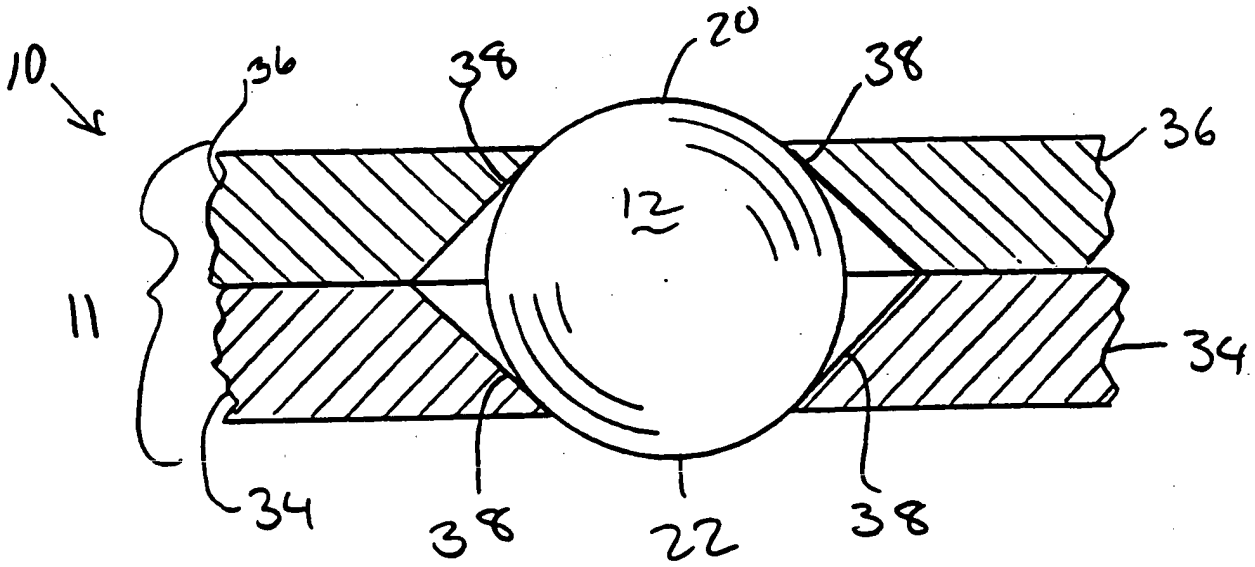


Fig. 7

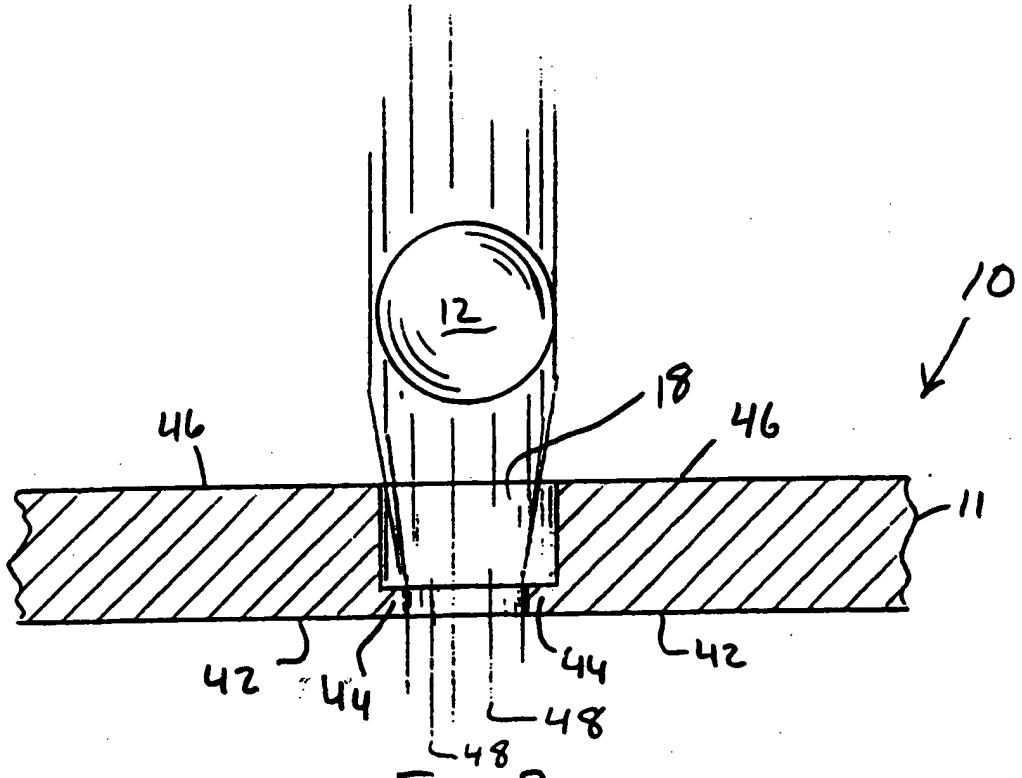


Fig. 8

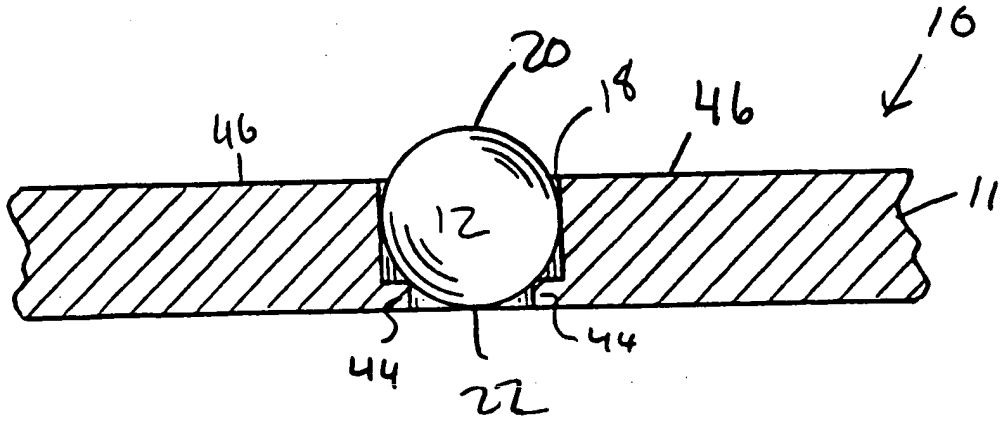


Fig. 9

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/23697

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :Please See Extra Sheet.
US CL :436/518, 524, 525, 526, 527, 528, 529, 530, 531, 54; 422/104
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 436/518, 524, 525, 526, 527, 528, 529, 530, 531, 54; 422/104

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, CASONLINE
search terms: combinatorial chemistry, combinatorial synthesis, resin beads, beads holder

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 4,470,954 A (CHIKNAS) 11 September 1984, see entire document | 1-20 |
| A | US 4,977,077 A (NGO et al.) 11 December 1990, see column 5, lines 10-29. | 1-20 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/23697

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 33/543, 33/544, 33/545, 33/551, 33/553, 33/554, 35/08; B01L 9/00